

SEQUENCE LISTING

<pre><110> Leo G.J. FRENKEN</pre>
Martine E. VERHOEYEN Joy E. WILKINSON Stephen A. JOBLING
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ggt cac tat ggt atg ggc tgg ttc cgc cag gtt cca ggg aag gag cgt Gly His Tyr Gly Met Gly Trp Phe Arg Gln Val Pro Gly Lys Glu Arg 35 40 45
gag ttt gtc gca gct att agg tgg agt ggt aaa gag aca tgg tat aaa 193 Glu Phe Val Ala Ala Ile Arg Trp Ser Gly Lys Glu Thr Trp Tyr Lys 50 55 60

gac tcc gtg a Asp Ser Val 1 65					
acg gtt tat o					
tat tat tgt of Tyr Tyr Cys I					
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Gly His Tyr (Gly Met Gly	Trp Phe Arg	Gln Val Pro	Gly Lys Gl	u Arg
Glu Phe Val 7	Ala Ala Ile	Arg Trp Ser 55	Gly Lys Glu 60	Thr Trp Ty	r Lys
Asp Ser Val 1	Lys Gly Arg 70	Phe Thr Ile	Ser Arg Asp 75	Asn Ala Ly	s Thr 80
Thr Val Tyr	Leu Gln Met 85	Asn Ser Leu	Lys Pro Glu 90	Asp Thr Ala	a Val
		Pro Val Arg	Val Asp Asp	Ile Ser Le	u Pro
Tyr Tyr Cys i	Ala Ala Arg 100	105		110	

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1 10 15

tct ctg aga ctc tcc tgt gta gct tct gaa agc agc ttc agc aac aat 96 Ser Leu Arg Leu Ser Cys Val Ala Ser Glu Ser Ser Phe Ser Asn Asn 20 25 30

cac atg ggc tgg tac cgc cgg gct cca ggg aac cag cgc gag ctg gtc 144 His Met Gly Trp Tyr Arg Arg Ala Pro Gly Asn Gln Arg Glu Leu Val 35 40 45

gca act att agt cct ggt ggt agc aca cac tat gta gac tcc gtg aag 192 Ala Thr Ile Ser Pro Gly Gly Ser Thr His Tyr Val Asp Ser Val Lys 50 55 60

ggc cga ttc acc atc tcc cga gac aac gcc aag aac aca gtg tat cta Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu 65 70 75 80	240
caa atg gac agc ctg aaa cca gag gac acg gcc gtc tat tac tgt gct Gln Met Asp Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala 85 90 95	288
gcc aag ggg agg ggg ctg cag gct atg cag tac tgg ggc cag ggg acc Ala Lys Gly Arg Gly Leu Gln Ala Met Gln Tyr Trp Gly Gln Gly Thr 100 105 110	336
ctg gtc acc gtc tcc tca gcg cac cac agc gaa gac ccc agc tcc gcg Leu Val Thr Val Ser Ser Ala His His Ser Glu Asp Pro Ser Ser Ala 115 120 125	384
gcc gcc cat cac cat cac cat cac ggg gcc gca gaa caa aaa ctc atc Ala Ala His His His His His Gly Ala Ala Glu Gln Lys Leu Ile 130 135 140	432
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Ser Leu Arg Leu Ser Cys Val Ala Ser Glu Ser Ser Phe Ser Asn Asn 20 25 30 His Met Gly Trp Tyr Arg Arg Ala Pro Gly Asn Gln Arg Glu Leu Val	
Ser Leu Arg Leu Ser Cys Val Ala Ser Glu Ser Ser Phe Ser Asn Asn 20 His Met Gly Trp Tyr Arg Arg Ala Pro Gly Asn Gln Arg Glu Leu Val 35 Ala Thr Ile Ser Pro Gly Gly Ser Thr His Tyr Val Asp Ser Val Lys	
Ser Leu Arg Leu Ser Cys Val Ala Ser Glu Ser Ser Phe Ser Asn Asn 20 His Met Gly Trp Tyr Arg Arg Ala Pro Gly Asn Gln Arg Glu Leu Val 35 Ala Thr Ile Ser Pro Gly Gly Ser Thr His Tyr Val Asp Ser Val Lys 50 Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu	



Leu Val Thr Val Ser Ser Ala His His Ser Glu Asp Pro Ser Ser Ala 120 Ala Ala His His His His His Gly Ala Ala Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Gly Ala Ala <210> 6 <211> 468 <212> DNA <213> Artificial <220> <223> VHH with linker <220> <221> CDS <222> (1)..(456) <400> 6 cag gtg cag ctg cag gag tet ggg gga gge etg gtg cag get ggg ggg 48 Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly 10 tct ctg aga ctc tcc tgt gta gcc tct gga aac acc ttc agt atc ata 96 Ser Leu Arg Leu Ser Cys Val Ala Ser Gly Asn Thr Phe Ser Ile Ile get atg gee tgg tae ege cag get eea ggg aag eag ege gag gte 144 Ala Met Ala Trp Tyr Arg Gln Ala Pro Gly Lys Gln Arg Glu Val Val gca agt att aat agt att ggc agc aca aat tat gca gac tcc gtg aag 192 Ala Ser Ile Asn Ser Ile Gly Ser Thr Asn Tyr Ala Asp Ser Val Lys 55 ggg cga ttc acc atc tcc aga gac aac gcc aag aac aca gtg tat ctg 240 Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu caa atg age age ctg aaa eet gag gac acg gee gte tat tae tgt get 288 Gln Met Ser Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala 90 gcc ggt aat ttg ctg gtt aag agg cct tac tgg ggc cag ggg acc ctg 336 Ala Gly Asn Leu Leu Val Lys Arg Pro Tyr Trp Gly Gln Gly Thr Leu 384 gtc acc gtc tcc tca gaa ccc aag aca cca aaa cca caa cca gcg gcc Val Thr Val Ser Ser Glu Pro Lys Thr Pro Lys Pro Gln Pro Ala Ala

120

432 gcc cat cac cat cac cat cac ggg gcc gca gaa caa aaa ctc atc tca Ala His His His His His Gly Ala Ala Glu Gln Lys Leu Ile Ser 130 135 gaa gag gat ctg aat ggg gcc gca tagtaacaat tg 468 Glu Glu Asp Leu Asn Gly Ala Ala 150 <210> 7 <211> 152 <212> PRT <213> Artificial <220> <223> VHH with linker <400> 7 Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys Val Ala Ser Gly Asn Thr Phe Ser Ile Ile Ala Met Ala Trp Tyr Arg Gln Ala Pro Gly Lys Gln Arg Glu Val Val Ala Ser Ile Asn Ser Ile Gly Ser Thr Asn Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu Gln Met Ser Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala Gly Asn Leu Leu Val Lys Arg Pro Tyr Trp Gly Gln Gly Thr Leu 100 Val Thr Val Ser Ser Glu Pro Lys Thr Pro Lys Pro Gln Pro Ala Ala 120 Ala His His His His His Gly Ala Ala Glu Gln Lys Leu Ile Ser 130 Glu Glu Asp Leu Asn Gly Ala Ala 150 <210> 8

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gct ggg ggc cct ctg agg ctc tcc tgt gca gcc tct gga cgc acc ttc Ala Gly Gly Pro Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe 20 25 30	96
agt aac tat gcc gtg ggc tgg ttc cgc cag gct cca ggg aag gag cgt Ser Asn Tyr Ala Val Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg 35 40 45	144
gag ttt gtc gct gct att agc cgt gat ggt ggg cgc aca tac tat gcg Glu Phe Val Ala Ala Ile Ser Arg Asp Gly Gly Arg Thr Tyr Tyr Ala 50 55 60	192
gac tcc gtg aag ggc cga ttc gcc gtc tcc aga gac tac gcc gag aac Asp Ser Val Lys Gly Arg Phe Ala Val Ser Arg Asp Tyr Ala Glu Asn 65 70 75 80	240
acg gtg tat ctg caa atg aac agc ctg aaa cct gag gac acg gcc gtt Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val 85 90 95	288
tat tac tgt aac aca agg gcc tac tgg ggc cag ggg acc cag gtc acc Tyr Tyr Cys Asn Thr Arg Ala Tyr Trp Gly Gln Gly Thr Gln Val Thr 100 105 110	336
gtc tcc tca gcg cac cac agc gaa gac ccc agc tcc gcg gcc gcc cat Val Ser Ser Ala His His Ser Glu Asp Pro Ser Ser Ala Ala Ala His 115 120 125	384
cac cat cac cat cac ggg gcc gca gaa caa aaa ctc atc tca gaa gag His His His His Gly Ala Ala Glu Gln Lys Leu Ile Ser Glu Glu 130 135 140	432
gat ctg aat ggg gcc gca tagtaacaat tg Asp Leu Asn Gly Ala Ala 145 150	462
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Ser Asn Tyr Ala Val Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg 35 40 45

Glu Phe Val Ala Ala Ile Ser Arg Asp Gly Gly Arg Thr Tyr Tyr Ala 50 60

Asp Ser Val Lys Gly Arg Phe Ala Val Ser Arg Asp Tyr Ala Glu Asn 65 70 75 80

Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val 85 90 95

Tyr Tyr Cys Asn Thr Arg Ala Tyr Trp Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser Ala His His Ser Glu Asp Pro Ser Ser Ala Ala His 115 120 125

His His His His Gly Ala Ala Glu Gln Lys Leu Ile Ser Glu Glu
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Asp Leu Asn Gly Ala Ala 145 150

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	ag ttt lu Phe 50														192
A	ac tcc sp Ser														240
	cg gtg hr Val			ln Met											288 _/
	at tac 'yr Tyr	Cys I													336
	tc tcc al Ser														384
	ac cat is His 130				_	_	_						_		432
A	at ctg sp Leu 45				Asp			tgat	caaca	aat t	g			•	471
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Ser As	n Tyr 35	Ala	Val	Gly	Trp	Phe 40	Arg	Gln	Ala	Pro	Gly 45	Lys	Glu	Arg	
Glu Ph 50		Ala	Ala	Ile	Ser 55	Arg	Asp	Gly	Gly	Arg 60	Thr	Tyr	Tyr	Ala	
Asp Se 65	r Val	Lys	Gly	Arg 70	Phe	Ala	Val	Ser	Arg 75	Asp	Tyr	Ala	Glu	Asn 80	
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Tyr Ty	r Cys	Asn 100	Thr	Arg	Ala	Tyr	Trp 105	Gly	Gln	Gly	Thr	Gln 110	Val	Thr	
Val Se	r Ser 115	Ala	His	His	Ser	Glu 120	Asp	Pro	Ser	Ser	Ala 125	Ala	Ala	His	
His Hi 13		His	His	Gly	Ala 135	Ala	Glu	Gln	Lys	Leu 140	Ile	Ser	Glu	Glu	
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Met Gly 1 Thr Let	/ Phe	Leu	5				Ser	10				Ala	15		
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act tca cta gac acc aaa tca acc ttg tca cag ata gga ctc agg aac Thr Ser Leu Asp Thr Lys Ser Thr Leu Ser Gln Ile Gly Leu Arg Asn 20 25 30	95
cat act ctg act cac aat ggt tta agg gct gtt aac aaa ctt gat ggg His Thr Leu Thr His Asn Gly Leu Arg Ala Val Asn Lys Leu Asp Gly 35 40 45	143
ctc caa tca aga act aat act aag gta aca ccc aag atg gca tcc aga Leu Gln Ser Arg Thr Asn Thr Lys Val Thr Pro Lys Met Ala Ser Arg 50 55 60	191
act gag acc aag aga cct gga tgc tca gct acc att gtt tgt gga aaa Thr Glu Thr Lys Arg Pro Gly Cys Ser Ala Thr Ile Val Cys Gly Lys 65 70 75	239
cag gtg cag ctg cag Gln Val Gln Leu Gln 80	254
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Glu Thr Lys Arg Pro Gly Cys Ser Ala Thr Ile Val Cys Gly Lys Gln
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Val Gln Leu Gln
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_	10	
	c tct ctg aga ctc tcc tgt gca gcc tcg gga cgc gcc acc agt y Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ala Thr Ser 20 25 30	95
	t ggt cac tat ggt atg ggc tgg ttc cgc cag gtt cca ggg aag s Gly His Tyr Gly Met Gly Trp Phe Arg Gln Val Pro Gly Lys 35 40 45	143

		gag Glu 50		_	-			-		_			-			191
		gac Asp														239
		acg Thr														287
		tat Tyr														335
		gtt Val														383
		gaa Glu 130		_												431
		ccc Pro					_		_	_			_		_	479
	_	ctc Leu														527
		gtc Val														575
_	_	gtg Val		_	_	_			_	_						623
		gca Ala 210														671
		agc Ser														719
_		ctg Leu	_						_	_	_				•	767
	_	gcc Ala				_				_	_			_		815
		ccg Pro	_			_	_	_				_		_	-	863

aag gac acc gtg agc gta acc tgc ctg gtc aaa ggc ttc tac cca cct Lys Asp Thr Val Ser Val Thr Cys Leu Val Lys Gly Phe Tyr Pro Pro 290 295 300	911
gat atc aac gtt gag tgg cag agg aac ggt cag ccg gag tca gag ggc Asp Ile Asn Val Glu Trp Gln Arg Asn Gly Gln Pro Glu Ser Glu Gly 305 310 315	959
acc tac gcc acc acg cca ccc cag ctg gac aac gac ggg acc tac ttc Thr Tyr Ala Thr Thr Pro Pro Gln Leu Asp Asn Asp Gly Thr Tyr Phe 320 325 330 335	1007
ctc tac agc aag ctc tcg gtg gga aag aac acg tgg cag cgg gga gaa Leu Tyr Ser Lys Leu Ser Val Gly Lys Asn Thr Trp Gln Arg Gly Glu 340 345 350	1055
acc ttc acc tgt gtg gtg atg cac gag gcc ctg cac aac cac tac acc Thr Phe Thr Cys Val Val Met His Glu Ala Leu His Asn His Tyr Thr 355 360 365	1103
cag aaa tcc atc acc cag tct tcg ggt aaa taa taagaattcg agctcgaa Gln Lys Ser Ile Thr Gln Ser Ser Gly Lys 370 375	1154
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Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ala Thr Ser Gly 20 25 30	
His Gly His Tyr Gly Met Gly Trp Phe Arg Gln Val Pro Gly Lys Glu 35 40 45	
Arg Glu Phe Val Ala Ala Ile Arg Trp Ser Gly Lys Glu-Thr Trp Tyr 50 55 60	
Lys Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys	·
65 70 75 80	
Thr Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala 85 90 95	
Thr Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala	

Ser Glu Pro Lys Thr Pro Lys Pro Gln Pro Gln Pro Gln Pro I30 135 140

1

Gln Pro Asn Pro Thr Thr Glu Ser Lys Cys Pro Lys Cys Pro Ala Pro 145 150 155 160

Glu Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Pro Lys
165 170 175

Asp Val Leu Ser Ile Ser Gly Arg Pro Glu Val Thr Cys Val Val 180 185 190

Asp Val Gly Gln Glu Asp Pro Glu Val Ser Phe Asn Trp Tyr Ile Asp 195 200 205

Gly Ala Glu Val Arg Thr Ala Asn Thr Arg Pro Lys Glu Glu Gln Phe 210 215 220

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Pro Ile Gln His Gln Asp 225 230 235 240

Trp Leu Thr Gly Lys Glu Phe Lys Cys Lys Val Asn Asn Lys Ala Leu 245 250 255

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Thr Arg
260 265 270

Glu Pro Gln Val Tyr Ala Leu Ala Pro His Arg Glu Glu Leu Ala Lys 275 280 285

Asp Thr Val Ser Val Thr Cys Leu Val Lys Gly Phe Tyr Pro Pro Asp 290 295 300

Ile Asn Val Glu Trp Gln Arg Asn Gly Gln Pro Glu Ser Glu Gly Thr 305 310 315 320

Tyr Ala Thr Thr Pro Pro Gln Leu Asp Asn Asp Gly Thr Tyr Phe Leu 325 330 335

Tyr Ser Lys Leu Ser Val Gly Lys Asn Thr Trp Gln Arg Gly Glu Thr 340 345 350

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ttc aac agc acg tac cgc gtg gtc agc gtc ctg ccc atc cag cac cag Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Pro Ile Gln His Gln 225 230 235
gac tgg ctg acg ggg aaa gag ttc aaa tgc aag gtc aac aac aaa gct Asp Trp Leu Thr Gly Lys Glu Phe Lys Cys Lys Val Asn Asn Lys Ala 240 255 255
ctc ccg gcc ccc atc gag aag acc atc tcc aag gcc aaa ggg cag acc 815 Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Thr 260 265 270
cgg gag ccg cag gtg tac gcc ctg gcc cca cac cgg gaa gag ctg gcc 863 Arg Glu Pro Gln Val Tyr Ala Leu Ala Pro His Arg Glu Glu Leu Ala 275 280 285
aag gac acc gtg agc gta acc tgc ctg gtc aaa ggc ttc tac cca cct Lys Asp Thr Val Ser Val Thr Cys Leu Val Lys Gly Phe Tyr Pro Pro 290 295 300
gat atc aac gtt gag tgg cag agg aac ggt cag ccg gag tca gag ggc Asp Ile Asn Val Glu Trp Gln Arg Asn Gly Gln Pro Glu Ser Glu Gly 305 310 315
acc tac gcc acc acg cca ccc cag ctg gac aac gac ggg acc tac ttc Thr Tyr Ala Thr Thr Pro Pro Gln Leu Asp Asn Asp Gly Thr Tyr Phe 320 325 330 335
ctc tac agc aag ctc tcg gtg gga aag aac acg tgg cag cgg gga gaa 1055 Leu Tyr Ser Lys Leu Ser Val Gly Lys Asn Thr Trp Gln Arg Gly Glu 340 345 350
acc ttc acc tgt gtg gtg atg cac gag gcc ctg cac aac cac tac acc Thr Phe Thr Cys Val Val Met His Glu Ala Leu His Asn His Tyr Thr 355 360 365
cag aaa tcc atc acc cag tct tcg ggt aaa tct gag aaa gat gag cta Gln Lys Ser Ile Thr Gln Ser Ser Gly Lys Ser Glu Lys Asp Glu Leu 370 375 380
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Tyr Ala Thr Thr Pro Pro Gln Leu Asp Asn Asp Gly Thr Tyr Phe Leu 325 330 Tyr Ser Lys Leu Ser Val Gly Lys Asn Thr Trp Gln Arg Gly Glu Thr 345 Phe Thr Cys Val Val Met His Glu Ala Leu His Asn His Tyr Thr Gln 360 Lys Ser Ile Thr Gln Ser Ser Gly Lys Ser Glu Lys Asp Glu Leu 375 <210> 58 <211> 31 <212> DNA <213> Artificial <220> <223> PCR primer <400> 58 31 aggggaccca ggtcaccgtc tcctcagaac c <210> 59 <211> 37 <212> DNA <213> Artificial <220> <223> PCR primer <400> 59 37 gagetttgtt gttgacettg catttgaact etttece <210> 60 <211> 26 <212> DNA <213> Artificial <220>

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	g ggg tot otg agg oto too tgt goa goo tot gga ago att tto y Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ser Ile Phe 20 25 30	95

aga cgt ccg cat atg ggt tgg ttc cgc cag gct cca ggg cag gag cgc Arg Arg Pro His Met Gly Trp Phe Arg Gln Ala Pro Gly Gln Glu Arg 35 40 45	143
gag ttg gtc gca ctg att tct gcg ggt ggt cgt aca tgg tat gca gac Glu Leu Val Ala Leu Ile Ser Ala Gly Gly Arg Thr Trp Tyr Ala Asp 50 . 55 60	191
tcc gtg aag ggc cga ttc acc atc tcc aga gac aac gcc aag aac acg Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr 65 70 75	239
ctg tat ctg caa atg aac agc ctg aaa cct gag gac acg gcc gtt tat Leu Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr 80 85 90 95	287
tac tgt act gcc ggg ggt tcg tac tgg ggc cag ggg acc cag gtc acc Tyr Cys Thr Ala Gly Gly Ser Tyr Trp Gly Gln Gly Thr Gln Val Thr 100 105 110	335
gtc gcc tca gaa ccc aag aca cca aaa cca caa cca gcg gcc gcc	383
cac cat cac cat cac ggg gcc gca gaa caa aaa ctc atc tca gaa gag His His His His Gly Ala Ala Glu Gln Lys Leu Ile Ser Glu Glu 130 135 140	431
gat ctg aat ggg gcc gca tagtaacaat tg Asp Leu Asn Gly Ala Ala 145	461
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Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ser Ile Phe Arg 20 25 30	
Arg Pro His Met Gly Trp Phe Arg Gln Ala Pro Gly Gln Glu Arg Glu 35 40 45	
Leu Val Ala Leu Ile Ser Ala Gly Gly Arg Thr Trp Tyr Ala Asp Ser 50 55 60	
Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu	

Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr 85 90 95

Cys Thr Ala Gly Gly Ser Tyr Trp Gly Gln Gly Thr Gln Val Thr Val 100 105 110

Ala Ser Glu Pro Lys Thr Pro Lys Pro Gln Pro Ala Ala Ala His His 115 120 125

His His His Gly Ala Ala Glu Gln Lys Leu Ile Ser Glu Glu Asp 130 135 140

Leu Asn Gly Ala Ala 145